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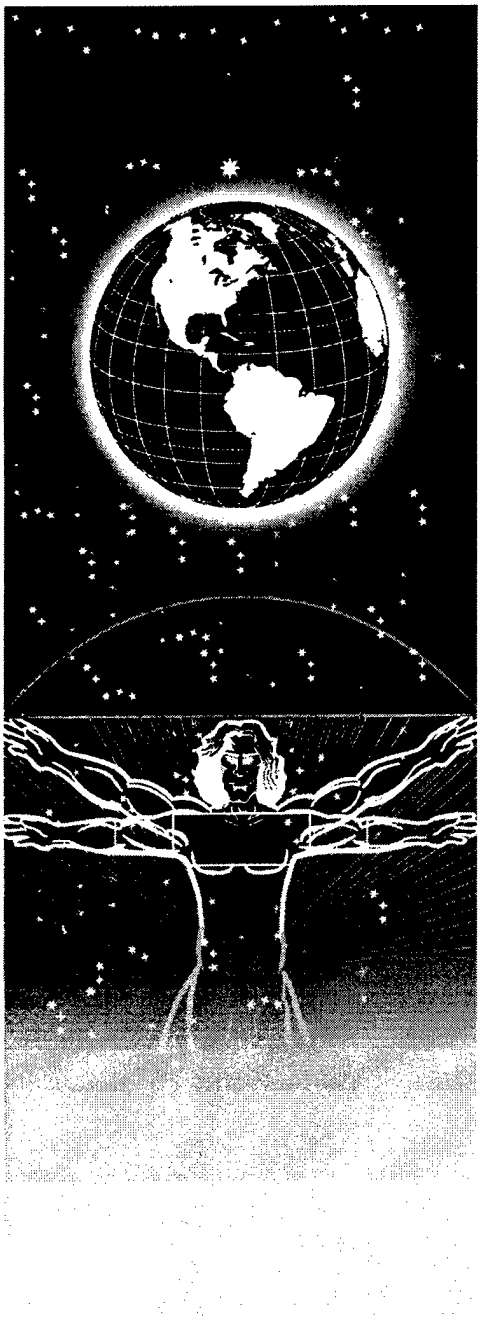

UNITED STATES AIR FORCE ARMSTRONG LABORATORY

EVIDENCE FOR INCREASED CARDIAC COMPLIANCE DURING EXPOSURE TO SIMULATED MICROGRAVITY

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13. ABSTRACT *(Maximum 200 words)*

We measured specific hemodynamic responses during 4 days (96 hours) of head-down tilt (HDT) in invasively-instrumented rhesus monkeys to test the hypothesis that exposure to simulated microgravity causes increased cardiac compliance. Six rhesus monkeys underwent the following two 5-day experimental conditions (days 0-4) separated by 9 days of ambulatory activities in a cross-over counterbalance design: 1) continuous exposure to 10ø HDT; and 2) 16 hours per day of 80ø head-up tilt and 8 hours supine (control). Each animal underwent daily measurements of central venous pressure (CVP), left ventricular (LVP) and aortic (AoP) pressures, stroke volume (SV), and esophageal pressure (EsP). Additionally, each animal underwent measurement of plasma volume (day 2) and provocative tests which included graded dose administration of phenylephrine (a1-adrenergic responsiveness) and isoproterenol (b1-adrenergic responsiveness) (day 3), and application of lower body negative pressure (day 4). Compared to the control condition, a 34% reduction in CVP (-1.6 mmHg, P = 0.010) and no change in left ventricular end-diastolic volume during HDT was associated with increased mean left ventricular end-diastolic compliance from 0.894 ñ 0.143 ml/mmHg compared to 1.111 ñ 0.170 ml/mmHg (P = 0.0053). Increased cardiac compliance could not be explained by reduced thoracic transmural pressure since EsP was unaltered by HDT. Our data provide the first direct evidence that increased cardiac compliance is associated with headward fluid shifts similar to those induced by exposure to spaceflight.

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Evidence for increased cardiac compliance during exposure to simulated microgravity

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Koenig, Steven C., Victor A. Convertino, John W. Fanton, Craig A. Reister, F. Andrew Gaffney, David A. Ludwig, Vladimir P. Krotov, Eugene V. Trambovetsky, and Rickey D. Latham. Evidence for increased cardiac compliance during exposure to simulated microgravity. *Am. J. Physiol.* 275 (*Regulatory Integrative Comp. Physiol.* 44): R 1 -R2, 1998.—We measured hemodynamic responses during 4 days of head-down tilt (HDT) and during graded lower body negative pressure (LBNP) in invasively instrumented rhesus monkeys to test the hypotheses that exposure to simulated microgravity increases cardiac compliance and that decreased stroke volume, cardiac output, and orthostatic tolerance are associated with reduced left ventricular peak dP/d*t*. Six monkeys underwent two 4-day (96 h) experimental conditions separated by 9 days of ambulatory activities in a crossover counterbalance design: *1*) continuous exposure to 10° HDT and *2*) ~12–14 h per day of 80° head-up tilt and 10–12 h supine (control condition). Each animal underwent measurements of central venous pressure (CVP), left ventricular and aortic pressures, stroke volume, esophageal pressure (EsP), plasma volume, α_1- and β_1-adrenergic responsiveness, and tolerance to LBNP. HDT induced a hypovolemic and hypoadrenergic state with reduced LBNP tolerance compared with the control condition. Decreased LBNP tolerance with HDT was associated with reduced stroke volume, cardiac output, and peak dP/d*t*. Compared with the control condition, a 34% reduction in CVP ($P = 0.010$) and no change in left ventricular end-diastolic area during HDT was associated with increased ventricular compliance ($P = 0.0053$). Increased cardiac compliance could not be explained by reduced intrathoracic pressure since EsP was unaltered by HDT. Our data provide the first direct evidence that increased cardiac compliance was associated with headward fluid shifts similar to those induced by exposure to spaceflight and that reduced orthostatic tolerance was associated with lower cardiac contractility.

head-down tilt; central venous pressure; adrenergic function; blood pressure

OBSERVATIONS FROM ACTUAL and ground-based analogs of spaceflight have revealed that central venous pressure (CVP) was reduced immediately on entry into orbit (2) and persisted throughout the mission (5, 6, 18). A paradoxical finding during the Spacelab Life Sciences (SLS-1 and -2) missions was that stroke volume and left ventricular end-diastolic dimensions during the first 2 days of spaceflight remained at or above preflight levels despite the reduction in filling pressure (2, 23). Because reduced CVP typically corresponds to a proportional reduction in cardiac filling (1), it has been proposed that the spaceflight observations may suggest an increase in cardiac compliance (2).

In addition to the potential for alterations in cardiac compliance, the impact of extended exposure to microgravity on cardiac function, particularly as it relates to orthostatic tolerance on return to earth, is unclear. Spaceflight and ground-based data have indicated that continued exposure to microgravity beyond 1 wk results in reduced stroke volume and cardiac output compared with supine 1-G control despite a compensatory elevation in heart rate (4, 6, 8, 9, 26). However, noninvasive measures of stroke volume (rebreathing techniques) and changes in cardiac size (echocardiography) have not provided sensitive indexes of cardiac contractility.

Direct assessment of cardiac compliance and contractility requires invasive measurement of changes in cardiac pressures and volumes. We therefore measured specific hemodynamic responses using an invasively instrumented rhesus monkey model to test the hypotheses that exposure to headward fluid shifts induced by a ground-based analog of microgravity causes increased cardiac compliance and that lowered stroke volume, cardiac output, and orthostatic tolerance are associated with reduced cardiac contractility.

METHODS

Subjects. Six mature male rhesus monkeys (*Macaca mulatta*) weighing 4.5–8 kg were selected as candidates for this study. All experimental procedures and protocols were reviewed and approved by the Institutional Animal Care and Use Committee at Brooks Air Force Base. The monkeys received 2 mo of tilt-table adaptation training before the experiments. This training involved three phases consisting of *1*) preliminary caretaker handling, restraint jacket fitting, and light ketamine sedation; *2*) acute restraint jacket and tilt-table acclimation training (<2 h); and *3*) increased restraint jacket and tilt-table adaptation training (up to 24 h).

After verification that monkeys were able to adapt to the tilt table during all phases of training, they were instrumented with chronically implanted right atrial and left ventricular access catheters, a transit-time flow probe encircling the ascending aorta, and pericardial leads (Fig. 1; Ref. 13). The catheters provided access sites for acute insertion of a single-tip 3F micromanometer (Millar Instruments, Houston, TX) into the right atrium for measurement of CVP and a double-tip 3F micromanometer (Millar Instruments) for measurement of left ventricular pressure (LVP) and aortic pressure (AoP) (14). An active redirection transit-time probe (Triton Technology, San Diego, CA) was used to measure

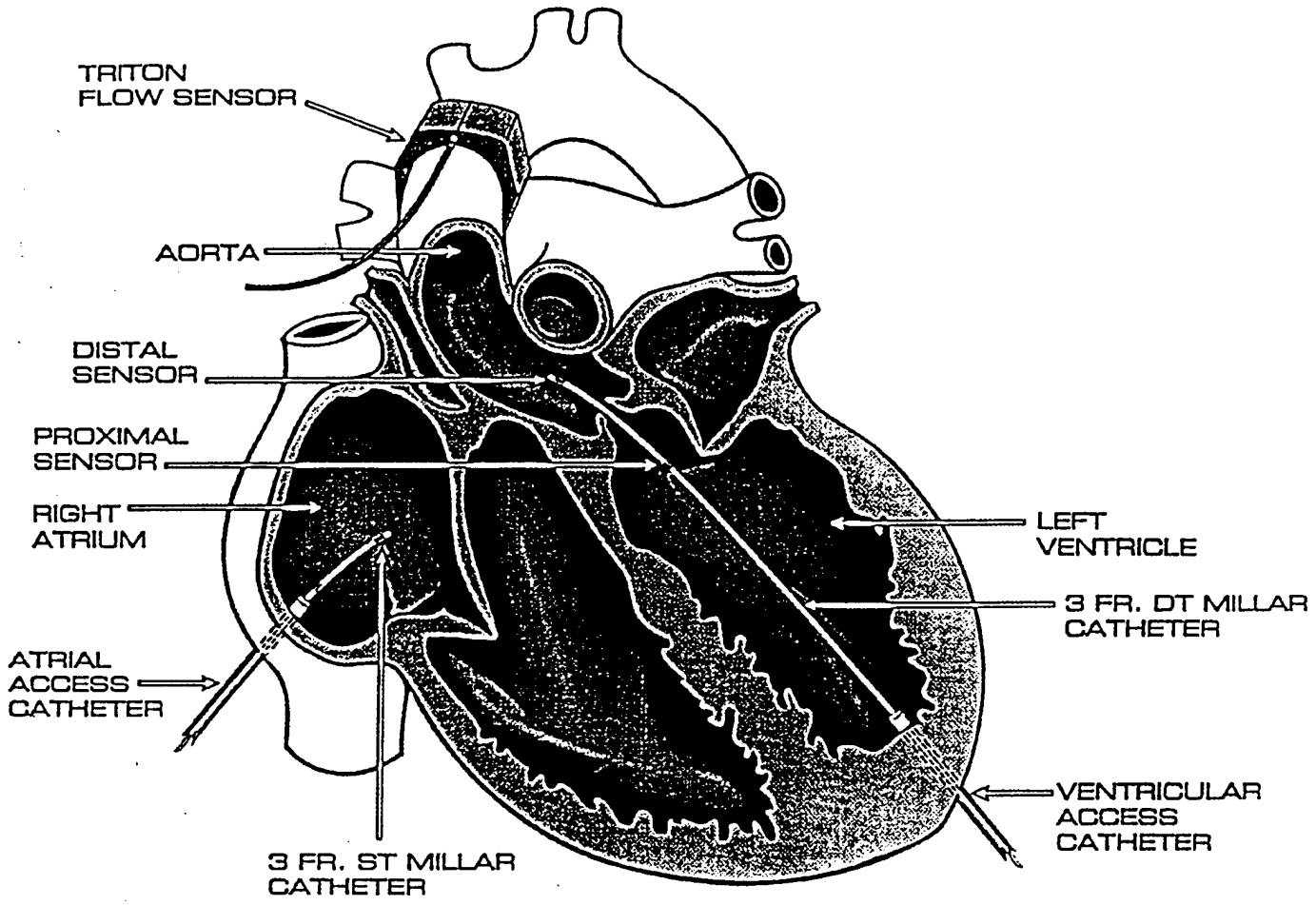

Fig. 1. Illustration of biosensor placement for chronic-implant model.

aortic blood flow (AoF). Left ventricular area was measured using transesophageal echocardiography (TEE) (Hewlett-Packard SONOS 1500). Esophageal pressure (EsP) was measured with a 5F disposable micromanometer (Millar Instruments), which was acutely inserted into the esophagus through a nostril so that the sensor was located at the same level as the heart. Electrocardiogram (ECG) was obtained using medical-grade stainless steel wire leads sutured to the visceral pericardium. Chronic implantation of biosensors was accomplished by median sternotomy. Subjects recovered ~1 mo postoperatively before the start of the test protocol.

Experimental design. A standard two-treatment crossover design was employed with each chronically instrumented rhesus monkey receiving both 10° head-down tilt (HDT) and upright/supine control conditions. The 10° HDT position was chosen because actual changes in cardiovascular responses induced by spaceflight have been closely simulated by this ground-based analog (4). The treatment order was randomized, but counterbalanced, so that three monkeys received HDT followed by the control condition and three monkeys received the control condition followed by HDT. Each treatment period lasted 96 h (4 days). The monkeys were kept unrestrained in their cages for a period of 9 days between treatment periods. An additional "days" effect independent of the treatment was introduced due to the measurement time course. Thus the experimental procedure consisted of two independent variables (treatment and time effects) over the sampling period. Daily 5-min hemodynamic recordings (baseline) were taken at approximately the same time every day (0915), with all measurements during control and HDT experimental conditions conducted in the 0° prone posture following visual observations of at least 5 min of stable hemodynamic responses to provide for comparisons of treatment effects. Before daily measurements of CVP, EsP, and TEE, an anesthetic (ketamine bolus of 30–50 mg iv) was administered to allow for insertion of the micromanometers and TEE probe, and the animals remained under anesthesia throughout baseline hemodynamic measures.

Measurement techniques. Selection of pressure and flow biosensors was made by evaluation of commercially available measurement systems and experimental studies (19, 20). Performance characteristics of pressure and flow sensors were tested in vitro in our cardiovascular dynamics simulation laboratory before chronic implantation. Atrial and ventricular access catheters were tested for patency, and the aortic flow probe and pericardial leads were tested for functional operation 2–3 wk postsurgery. During testing procedures, daily pre- and postcalibrations of esophageal and right atrial micromanometers, aortic flow probe, and ECG were accomplished. The dual-sensor aortic and left ventricular micromanometers were pre- and postcalibrated at the start and end of each 4-day treatment period. Physical calibrations of the pressure sensors were performed in a custom-fabricated calibration chamber using a digital manometer and positive and negative pressure pumps (Omega Engineering, Stamford, CT). Right atrial and esophageal sensors were calibrated from −15 to +25 mmHg, and aortic and left ventricular sensors were calibrated from −15 to +190 mmHg.

Electrical DC equivalent voltage calibrations were performed for ECG (0–1 mV) and aortic flow (0–8 l/min).

Signal conditioning of biosensor outputs was performed to amplify the low-level signals and maximize resolution of the input range of A/D converters, distribute amplified waveforms to data acquisition units, and accomplish antialias filtering. Pressure sensor outputs were amplified with fixed gain differential direct current amplifiers (Ectron, San Diego, CA). Aortic flow signal conditioning was accomplished using a commercial transit-time module (Triton Technology). ECG was measured using a Biotach ECG amplifier (Gould, Houston, TX). The conditioned analog signals were then fed into a custom-designed signal distribution unit, essentially a buffer amplifier, for channeling outputs to data acquisition units. The primary data acquisition unit was an A/D station comprised of antialaising filters (Precision Filters, Phoenix, AZ), A/D board (National Instruments, Austin, TX), desktop computer (Zeos, Nampa, ID), and A/D support software (National Instruments). Data were low-pass filtered at 60 Hz and sampled at 250 Hz. The secondary data acquisition units were a VHS analog recorder (Racal, Southampton, UK), an analog monitor (Gould), and a thermal array chart recorder (Astromed, West Warwick, RI).

Transesophageal echocardiography was performed with the use of a biplane pediatric probe kindly supplied for this study by Hewlett-Packard. A two-chamber view with maximal left ventricular area was recorded and analyzed offline using a commercial video digitizing system (Freeland Systems). Only area measurements are provided in this paper because of the lack of an acceptable method for calculating left ventricular volumes from two-dimensional TEE images in rhesus monkeys. Left ventricular areas provide only semiquantitative estimates of left ventricular volume, but should be adequate for identifying the presence or absence of significant intrasubject differences between HDT and control interventions.

Training and experimental sessions were conducted with subjects placed on custom-designed tilt tables positioned at one of three settings: 10° HDT, 0° prone, and 80° head-up tilt. Subjects were restrained to the table during all settings using custom-designed jackets that allowed full range of motion for arms, legs, and head, enabling subjects to feed themselves and minimizing adverse stress effects. The control condition lasted a total of 96 h (4 days) and consisted of 2 h of 80° head-up tilt (0700–0900), up to 4 h of 0° prone during experimental measurements (0900–1300), 10–12 h of 80° head-up tilt (1300–2300), and 8 h of 0° prone to simulate sleeping posture (2300–0700). The HDT treatment condition also lasted a total of 96 h (4 days) and consisted of up to 4 h of 0° prone during experimental measurements (0900–1300) and remaining time in 10° HDT (1300–0900).

Plasma volume measurement. Plasma volume was determined on *day 3* of HDT and control periods following baseline hemodynamic and TEE recordings by a modified dilution technique using sterile solutions of Evans blue dye contained in 10-ml ampules (The New World Trading, DeBary, FL). A preinjection control blood sample was drawn, followed by an intravenous injection of 11.5 mg of dye diluted with isotonic saline solution (2.5 ml), which was administered through a sterile 0.45-μm filter. One milliliter of plasma from a 10-min postinjection blood sample was passed through a wood-cellulose powder (Solka-Floc SW-40A) chromatographic column so that the dye could be absorbed. The absorbed dye was eluted from the column using a 1:1 water-acetone solution (pH = 7.0) and collected in a 10-ml volumetric flask. The postinjection solution was compared with 1-ml samples from a preinjection time (zero control) and a standard dye solution (1:50 dilution with distilled water), and all samples were read at 615 nm with a spectrophotometer. Using these procedures in our laboratory, test-retest correlation coefficient for plasma volume was 0.969 (n = 12) and the average changes were 82 ml [average change (Δ) = 1.5%, n = 17], 75 ml (average Δ = 1.5%, n = 19), and 56 ml (average Δ = 1.1%, n = 23) when measurements were determined 4, 8, and 15 days apart, respectively (16).

Adrenoreceptor tests. Measurement of adrenoreceptor responsiveness was performed on *day 3* of HDT and control periods. Following daily baseline hemodynamic recordings and recovery from anesthesia (i.e., regained consciousness), phenylephrine and isoproterenol infusion tests were conducted. Phenylephrine was infused at three graded constant rates (0.25, 0.50, and 1.00 $\mu g \cdot kg^{-1} \cdot min^{-1}$) using a syringe infusion pump (Baxter AS40A, Deerfield, IL). Each consecutive infusion interval lasted 9 min. Four 2-min hemodynamic recordings were made at baseline (preinfusion) and during the last 2 min at each infusion rate (*minutes 7–9, minutes 16–18, minutes 25–27*). A 30-min return to baseline period followed to allow effects of phenylephrine to subside. Isoproterenol was infused at three graded constant rates of 0.005, 0.01, and 0.02 $\mu g \cdot kg^{-1} \cdot min^{-1}$. The same experimental procedure and recordings used in the phenylephrine protocol were followed.

Lower body pressure. An acute lower body pressure (LBP) test was performed on *day 4* of HDT and control periods. After the daily hemodynamic recordings and while still under anesthesia, subjects were fitted with inflatable skirts and positioned inside an LBP chamber placed on the tilt table at 0° prone position. Sedation was continued with ketamine infused at a constant rate (500 $\mu g \cdot kg^{-1} \cdot min^{-1}$) with a syringe pump (Baxter AS40A, Deerfield, IL) and maintained throughout the duration of the LBP test. An esophageal catheter and TEE probe were then inserted. Ten graded levels of positive and negative pressure were applied to the chamber using an electromechanical control system invoking alterations in CVP and volume shifts. The pressure application sequence consisted of baseline (0 mmHg pressure) and graded steps from +30 mmHg down to −50 mmHg in 10-mmHg increments, with each pressure level lasting 3 min (total test time of 30 min). The first minute at each pressure level was allocated for subject acclimation. Hemodynamic data were then recorded for 30 s. During the last 90 s, TEE data were collected. The LBP test was subject to termination at the discretion of the attending medical monitor. The criterion for termination of the test was a mean aortic pressure below 50 mmHg. Hemodynamic measurements were used to calculate heart rate, stroke volume, cardiac output, mean aortic pressure, TPR, and peak-positive slope of the left ventricle (peak $LV_{dP/dt}$).

Data reduction. Hemodynamic data were analyzed using custom-designed software developed in Matlab (MathWorks, Natick, MA). This software enabled calculation of systolic, diastolic, and mean aortic pressure, left ventricular systolic and diastolic pressure, diastolic and end-systolic compliance, peak $LV_{dP/dt}$, mean central venous pressure, mean esophageal pressure, stroke volume, heart rate, and cardiac output from direct beat-to-beat measurements of CVP, AoP, LVP, EsP, heart rate, and AoF. Left ventricular diastolic compliance (LVdC), which we assumed to be constant during cardiac filling, was calculated by dividing the change in left ventricular volume by the change in transmural pressure between begin-diastole ($LVP_{bd} - EsP_{bd}$) and end-diastole ($LVP_{ed} - EsP_{ed}$) (*Eq. 1*). The change in volume during filling is by definition the stroke volume, which was calculated by integrating aortic flow. The change in transmural pressure was

defined as the pressure gradient across the ventricular wall, which was calculated as the difference between LVP and EsP

$$LVdC = \frac{SV}{[(LVP_{ed} - EsP_{ed}) - (LVP_{bd} - EsP_{bd})]} \quad (1)$$

Statistical methods. A standard 2 × 4 within-subjects (repeated measures) ANOVA was used to identify differences between HDT and control conditions. This model was constructed for each dependent variable and evaluated the main effect of treatment condition, the main effect of day, and the day-by-treatment interaction. Exact P values were calculated for each independent effect and reflect the probability of obtaining the observed effect given only sampling variability (chance model). When appropriate, P values associated with sources of variation involving days were adjusted with the Huynh and Feldt correction to account for the non-random error structure of time (17). In the event of interaction, separate treatment condition-by-day comparisons were obtained by calculating the least-significant difference (LSD) from ANOVA mean square error (MSE) estimates (3). The LSD provided the difference that must exist between HDT and control means before a statistical difference is obtained ($\alpha = 0.05$). Orthogonal polynomials were used to describe the general effect over days when day-to-day differences were detected. Pooled standard errors were calculated from MSE estimates and used in the formulation of statistical tests. Raw standard errors, specific to each treatment mean, are provided, but do not reflect variation specific to the experimental design or the variability associated with statistical tests. The same statistical modeling techniques were used for the adrenoreceptor and LBNP tests. For these tests, infusion rate and pressure replaced days as the second factor in the repeated-measures model.

RESULTS

Day-by-treatment means for CVP and left ventricular end-diastolic area are illustrated in Fig. 2. Across days, CVP was consistently lower by an average of 1.57 mmHg in the HDT condition compared with control [$F(1,5) = 16.53$, $P = 0.0097$]. There was a decline in CVP from *day 1* to *day 4* of 0.275 mmHg/day (Fig. 2*A*) across both treatment groups [$F(3,15) = 2.52$, $P = 0.0969$]. The response profile for both stroke volume and left ventricular end-diastolic area across days for each treatment condition was parallel, indicating no interaction between days and treatment conditions ($P > 0.50$). There was no overall HDT effect ($P > 0.50$) on baseline left ventricular end-diastolic area (Fig. 2*B*). Reductions in CVP were associated with less blood volume during HDT (613 ± 28 ml) compared with the control condition (646 ± 36 ml) as a result of a 10% lower plasma volume ($t = 2.998$, df = 5, $P = 0.0302$) during HDT (369 ± 15 ml) than during the control condition (408 ± 19 ml). Hematocrit was elevated in HDT (39.6 ± 0.8) compared with the control condition (36.5 ± 1.5).

Day-by-treatment means for heart rate, stroke volume, cardiac output, systolic and diastolic pressures of the left ventricle and aorta, and peak $LV_{dP/dt}$ are presented in Table 1. The HDT effect was constant across days for stroke volume and cardiac output, while heart rate differences between the HDT and control conditions diminished as days increased. Differences

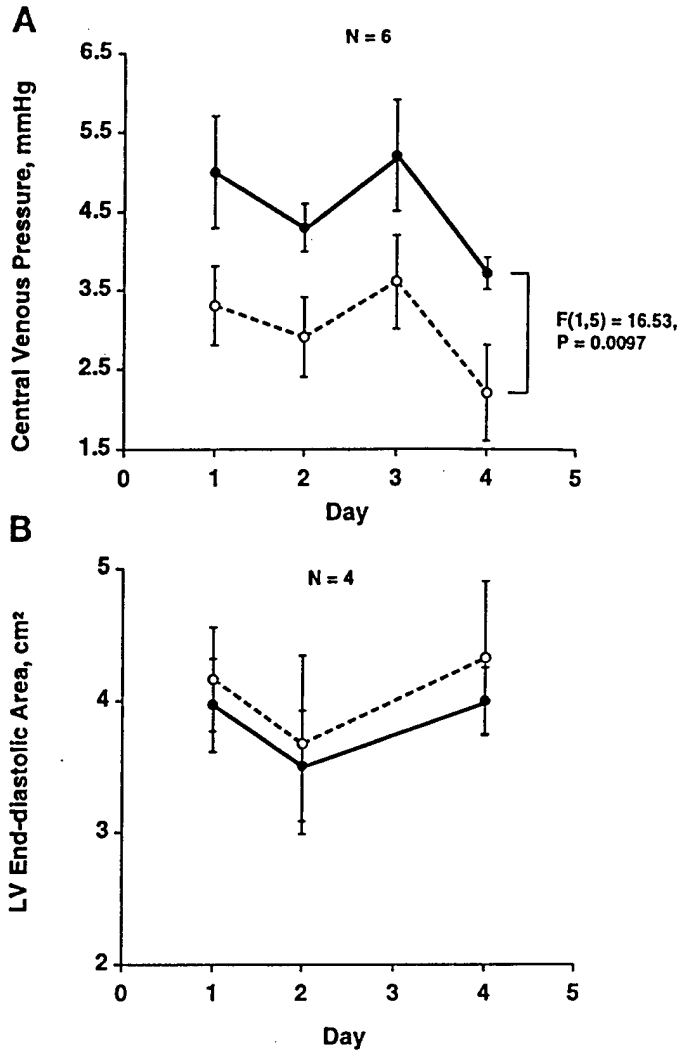

Fig. 2. Day-by-treatment comparison for central venous pressure and left ventricular (LV) end-diastolic area between 10° head-down tilt (HDT) (○) and control condition (●). Circles represent mean values and bars represent SE.

between the HDT and control conditions averaged 11 beats/min for heart rate [$F(1,5) = 12.61$, $P = 0.0164$], 1.1 ml for stroke volume [$F(1,5) = 4.26$, $P = 0.0938$], and 222 ml/min for cardiac output [$F(1,5) = 6.43$, $P = 0.0522$]. There was no apparent day effect for stroke volume ($P > 0.45$). Average linear decline across days was -4 beats·min^{-1}·day^{-1} for heart rate [$F(3,15) = 7.11$, $P = 0.0074$] and -37.2 ml·min^{-1}·day^{-1} for cardiac output [$F(3,15) = 3.28$, $P = 0.0789$]. Heart rate differences between the HDT and control conditions diminished during the final 2 days of the experiment [interaction: $F(3,15) = 3.45$, $P = 0.0483$]. The LSD value for heart rate was 9 beats/min. Differences in heart rate between the HDT and control conditions decreased as the experimental period progressed as a result of heart rate decreasing across days at a greater rate in the control than the HDT condition. Although the HDT condition showed consistently lower heart rates than the control condition, a floor effect in the HDT condition may have restricted the rate of day-to-day decline.

Table 1. *Hemodynamic responses on days 1–4 of control and HDT*

	Day 1	*Day 2*	*Day 3*	*Day 4*
Heart rate, beats/min				
Control	158 ± 4	153 ± 5	147 ± 4	142 ± 5
HDT	142 ± 7	138 ± 5	141 ± 7	134 ± 7
Stroke volume, ml				
Control	6.6 ± 1.4	6.4 ± 1.2	6.5 ± 1.0	6.3 ± 1.1
HDT	5.4 ± 0.7	5.4 ± 0.8	5.5 ± 0.6	5.1 ± 0.8
Cardiac output, ml/min				
Control	1,064 ± 224	986 ± 181	956 ± 151	902 ± 153
HDT	781 ± 124	758 ± 116	794 ± 100	694 ± 110
Total peripheral resistance				
Control	0.175 ± 0.084	0.156 ± 0.062	0.135 ± 0.035	0.146 ± 0.042
HDT	0.157 ± 0.039	0.172 ± 0.046	0.159 ± 0.035	0.199 ± 0.068
LV systolic pressure, mmHg				
Control	121 ± 5	118 ± 4	120 ± 4	119 ± 4
HDT	117 ± 6	119 ± 7	123 ± 7	119 ± 7
LV end-diastolic pressure, mmHg				
Control	7.5 ± 0.9	8.3 ± 1.5	8.3 ± 0.8	8.4 ± 0.8
HDT	4.5 ± 0.6	4.8 ± 0.6	5.0 ± 0.5	4.6 ± 0.5
LV begin-diastolic pressure, mmHg				
Control	0.0 ± 0.4	1.3 ± 1.2	1.1 ± 0.9	1.2 ± 0.7
HDT	−0.7 ± 0.4	−0.3 ± 0.3	0.1 ± 0.4	−0.2 ± 0.3
Aortic systolic pressure, mmHg				
Control	119 ± 5	116 ± 4	118 ± 4	117 ± 4
HDT	116 ± 6	118 ± 7	123 ± 6	118 ± 7
Aortic diastolic pressure, mmHg				
Control	83 ± 3	81 ± 4	81 ± 4	79 ± 3
HDT	79 ± 4	82 ± 4	85 ± 4	81 ± 4
LV dP/*dt*, mmHg/s				
Control	3,670 ± 390	3,303 ± 204	3,180 ± 199	2,951 ± 217
HDT	3,120 ± 305	2,851 ± 232	3,152 ± 191	2,783 ± 172

Values are means ± SE; $n = 6$. HDT, head-down tilt; LV, left ventricular.

Although baseline left ventricular diastolic pressure was consistently higher in the control condition than the HDT condition (3.4 mmHg), none of the left ventricular or aortic pressures showed any statistically discernible effects of treatment [$F(1,5) < 2.81$, $P > 0.1546$], days [$F(3,15) < 1.85$, $P > 0.1815$], or interaction [$F(3,15) < 1.91$, $P > 0.1713$]. $LV_{dP/dt}$ was reduced in the HDT condition compared with the control condition during *days 1* and *2*, whereas treatment differences were negligible during *days 3* and *4*. Although the LSD value of 385 mmHg/s suggested a differential effect, the test of interaction indicated that this differential effect was not that unlikely given only sampling variation [$F(3,15) = 1.63$, $P = 0.2200$]. As with heart rate, rate of change across days for the HDT condition may have been restricted due to lower overall values within this treatment group (floor effect). Overall, average $LV_{dP/dt}$ was 299 mmHg/s higher in the control condition than the HDT condition [$F(1,5) = 9.89$, $P = 0.0255$]. Averaged across treatment conditions, day-to-day decline in $LV_{dP/dt}$ averaged -149.5 mmHg·s^{-1}·day^{-1} [$F(3,15) = 6.90$, $P = 0.0108$]. The statistical analysis for a main effect of treatment on TPR revealed no overall HDT effect [$F(1,5) = 2.44$, $P = 0.1789$]. However, the difference in peripheral resistance between HDT and the control condition, specific to *day 4* (0.052 mmHg·min·ml^{-1}) was less than the LSD value of 0.055. There was no indication of a day effect or a day-by-treatment interaction for TPR ($P > 0.50$).

Day-by-treatment means for baseline EsP and left ventricular diastolic compliance are presented in Fig. 3. There were no statistically discernible differences across treatment conditions, days, or their interaction for mean EsP ($P > 0.30$). However, across days, left ventricular diastolic compliance was consistently higher in the HDT condition than the control condition by an average of 0.22 ml/mmHg [$F(1,5) = 22.18$, $P = 0.0053$]. No day-to-day variation or interaction were detected for left ventricular diastolic compliance ($P > 0.50$).

The dose-response relationships between the graded infusion levels of phenylephrine and TPR and isoproterenol and heart rate are presented in Fig. 4. The dose response of TPR during phenylephrine infusion was the same across both treatment conditions [$F(3,15) = 0.3633$, $P = 0.7805$]. Heart rate response to isoproterenol infusion was greater in the HDT than the control condition [$F(3,15) = 5.25$, $P = 0.0122$]. The linear component for the HDT condition was 1,720 beats/min/(μg·kg^{-1}·min^{-1}) compared with 960 beats/min/(μg·kg^{-1}·min^{-1}) for the control condition [$F(1,5) = 10.06$, $P = 0.0248$].

Mean values for stroke volume, cardiac output, heart rate, mean arterial pressure, TPR, and $LV_{dP/dt}$ during graded lower body negative pressure (LBNP) through −50 mmHg are presented in Fig. 5. Apart from the overall effect of HDT (i.e., profile height differences), LBNP dose-response functions were similar for both the control and HDT conditions ($P > 0.30$ for all dependent variables). It should be noted that following HDT, two subjects were unable to complete −40 mmHg LBNP and three others could not complete −50 mmHg LBNP. During control, two subjects were unable to

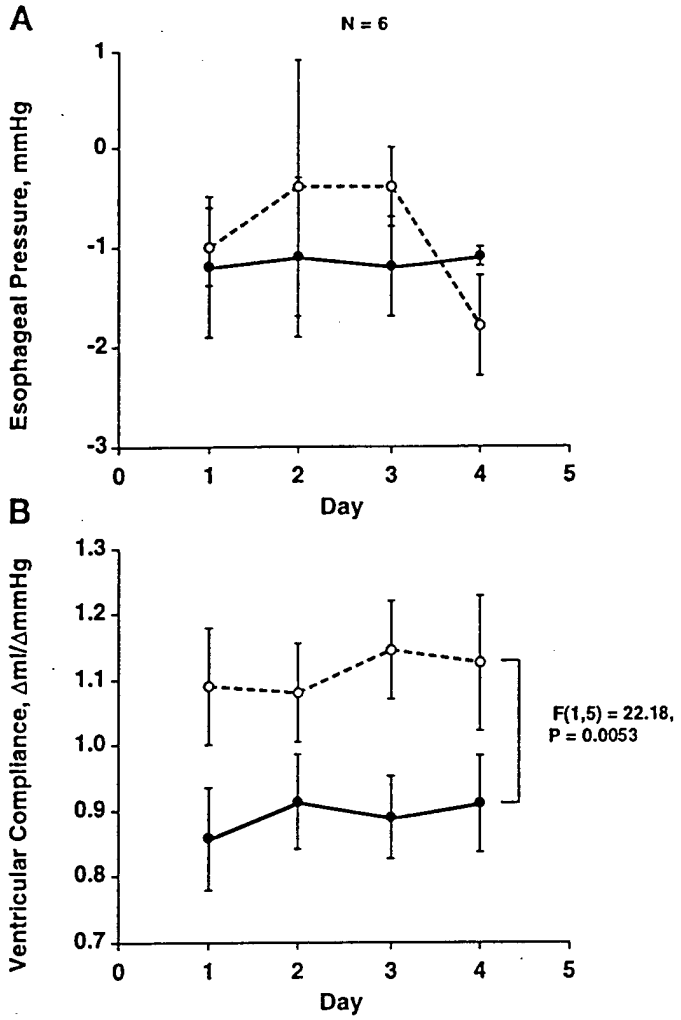

Fig. 3. Day-by-treatment comparison for mean esophageal pressure and left ventricular diastolic compliance between 10° HDT (O) and control condition (●). Circles represent mean values and bars represent SE. Δ, Change.

complete the −50 mmHg LBNP level. The high dropout rate at −50 mmHg prohibited the inclusion of these data in the statistical analysis. Because the general linear model was the basis for all the statistical analyses, adjustment due to missing data was inherent to the statistical methodology. Least-squares adjustments to account for missing data allows for the interpretation of the results as if the design were completely balanced.

DISCUSSION

In the present investigation, exposure to HDT caused a 34% reduction in CVP, a finding consistent with human spaceflight (2, 18) and ground-based data (5, 6, 26). Despite a reduced filling pressure in space, end-diastolic ventricular dimensions remained constant or slightly elevated during the initial day of spaceflight (23). We also observed no alteration in end-diastolic ventricular area in our monkeys despite a reduction in CVP. Under normal gravity, acute reductions in central blood volume and filling pressure induced in human subjects with the use of graded LBNP have demonstrated that a typical response relationship reflects a reduction in end-diastolic ventricular volume that is proportional to the reductions in CVP (1). Taken together, results from spaceflight and our study support the notion that exposure to microgravity alters the typical relationship between filling pressure and filling volume.

Maintained or increased transmural pressure across the myocardium could provide one possible mechanism for the paradoxical relationship between reduced filling pressure and maintained left ventricular end-diastolic area observed in the present investigation. If the headward fluid shift associated with HDT caused intrapleural pressure to decrease, transmural pressure would be maintained or increased, thereby maintaining or improving cardiac filling and left ventricular end-diastolic area. However, our results do not support this

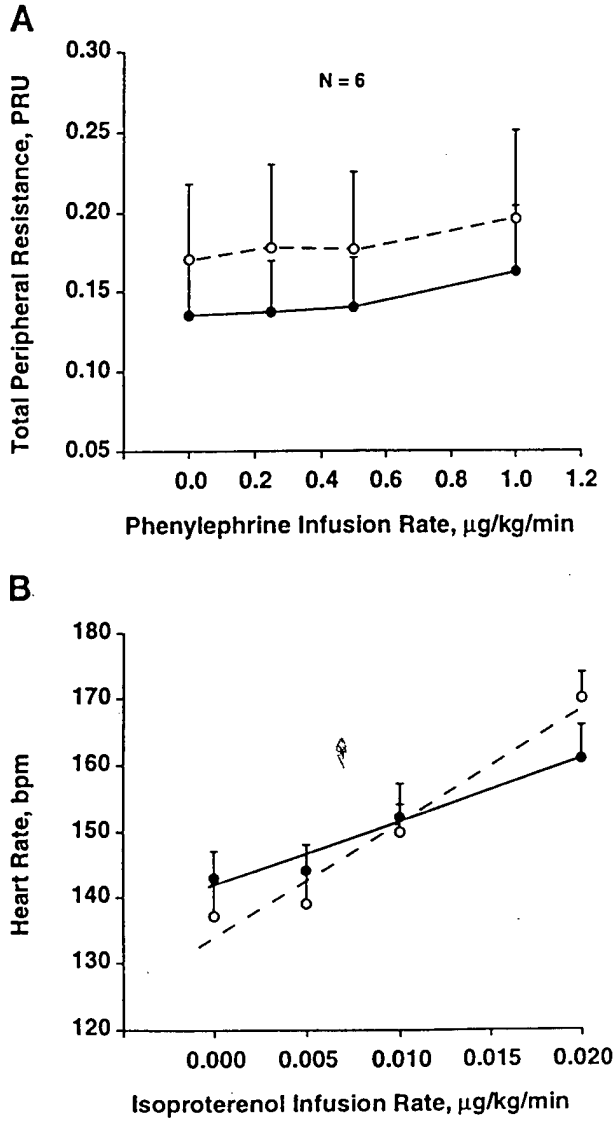

Fig. 4. Dose-response relationships between phenylephrine and total peripheral resistance (*A*) and isoproterenol and heart rate (*B*) during HDT (O) and control (●) treatments. Linear regressions are calculated from mean values. PRU, peripheral resistance units; bpm, beats/min.

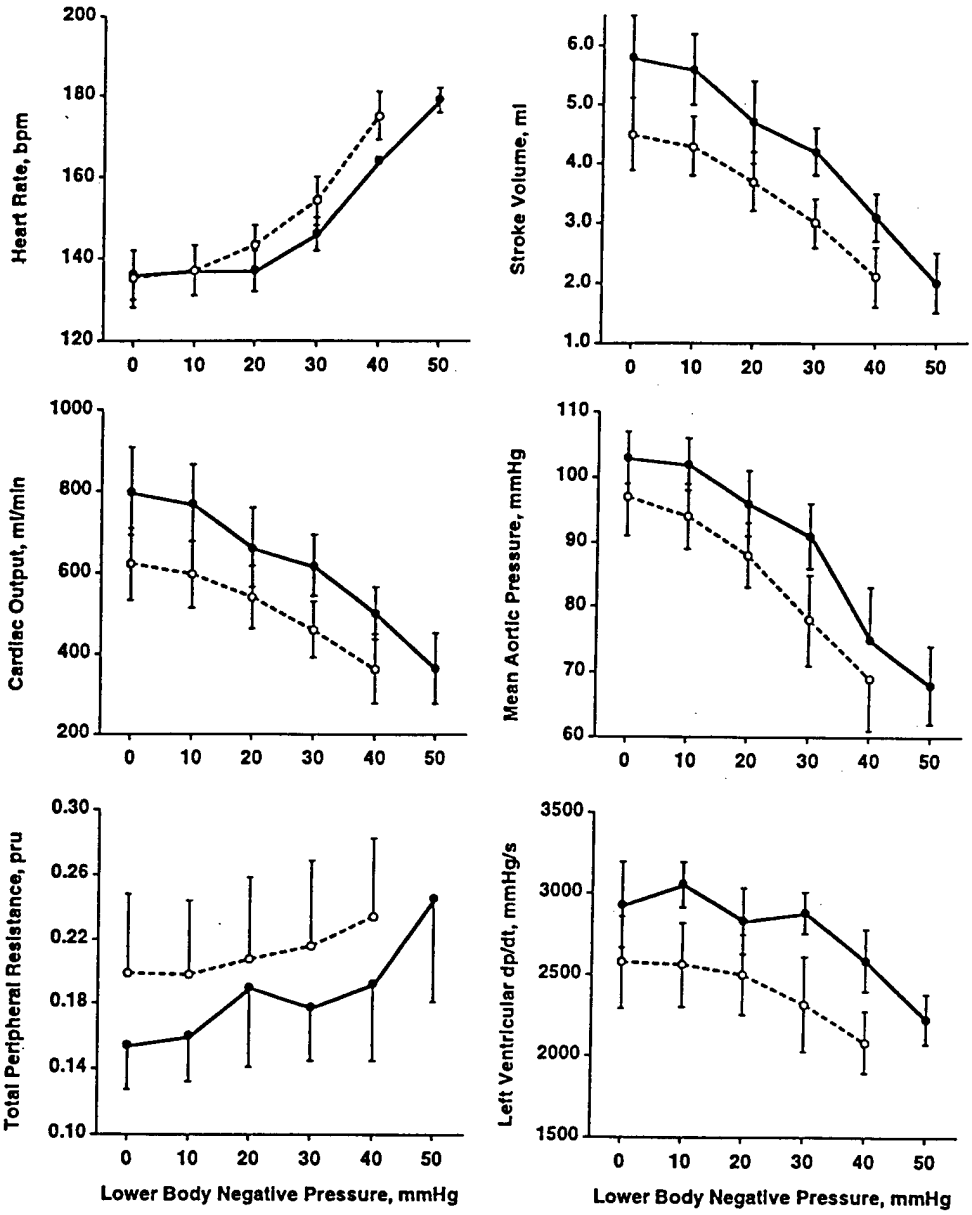

Fig. 5. Hemodynamic responses at 0 (baseline), 10, 20, 30, 40, and 50 mmHg LBNP during control (●) and HDT (○). Symbols represent mean ± SE of 6 animals.

hypothesis because there was no change in EsP between the control and HDT treatments in our animals.

In accordance with spaceflight data (2), stroke volume could be maintained or increased with less filling pressure if a hyperadrenergic state was induced that in turn resulted in increased ventricular contractility. Contrary to this possibility, evidence from the present study suggested that a ground analog of microgravity caused a hypoadrenergic state in our animals. A hypoadrenergic state is associated with low resting heart rate, low circulating catecholamines, and increased sensitivity of adrenergic receptors (24). Baseline heart rate was lower during HDT compared with control in the present study, a finding consistent with that observed in humans during short-duration spaceflight (15). Circulating catecholamines were not measured, but increased sensitivity of β_1-adrenoreceptors, as evidenced by greater heart rate responses during graded isoproterenol infusion following HDT compared with control, also suggests a hypoadrenergic state was present. Direct measurement of peak $LV_{dP/dt}$ suggested that left ventricular contractility was reduced (*days 1* and *2*) or maintained (*days 3* and *4*) during HDT compared with control at baseline and during LBNP (Fig. 5). It is recognized that peak dP/d*t* is affected by changes in arterial blood pressure (afterload) and, to a lesser extent, left ventricular filling (preload). However, systolic pressures were not changed by HDT compared with the control state. Also, left ventricular volume, as reflected by echocardiographic measurements of left ventricular area, was unchanged. Thus peak dP/d*t* should represent an appropriate estimate of left ventricular contractility in this study.

A major finding of this study was our direct evidence that a ground-based analog of microgravity induced a 26% increase in left ventricular diastolic compliance. Although increased cardiac compliance was proposed as a possible explanation for maintaining filling volume

in the face of reduced filling pressure during spaceflight (2) and preliminary results in rhesus monkeys have supported this notion (7), we are unaware of any previous experiments designed with direct measurements and appropriate control conditions that have tested this hypothesis. Left ventricular areas, as measured by transesophageal echocardiography, were unchanged, confirming that compliance was increased by HDT and was not merely a result of making measurements on the flatter (more compliant) region of an unchanged left ventricular compliance curve. The absence of alterations in left ventricular end-diastolic area with reduced filling pressure further substantiated the condition of increased cardiac compliance in our animals and eliminates the concern that the results were caused by taking points from different operating regions of the same compliance "curve."

Although the mechanism(s) underlying increased cardiac compliance during microgravity exposure are unclear, they may include mechanical factors associated with headward fluid shifts. It is possible that the reduction in CVP may be caused by increased pulmonary elasticity due to redistribution of blood away from the base to the apex of the lungs (11, 12, 23, 27). Reduced CVP caused by such pulmonary fluid redistribution and associated increases in left ventricular end-diastolic volume and/or stroke volume would then occur with an increase in effective cardiac compliance. In addition, it is conceivable that headward fluid shifts and increased left ventricular compliance during microgravity may be associated with a reduction in myocardial blood volume because reduced left ventricular compliance has been associated with increased myocardial blood volume and perfusion rates (25). The contribution of these mechanical factors to increased cardiac compliance during spaceflight requires further investigation.

Heart rate decreased by ~7% in HDT compared with the control condition in our animals. Our results are in close agreement with those of recent data from 12 astronauts who showed that average baseline resting heart rate decreased by 7% from 69 to 64 beats/min during 5 to 10 days of spaceflight (15). These findings are consistent with evidence in the present study and other experiments of a hypoadrenergic state. However, there are consistent observations that heart rate increases over time in microgravity compared with the supine position on earth (5, 6, 8, 9, 26). Chronotropic effects of exposure to microgravity may be represented by reductions in heart rate in the early stage of microgravity exposure (7–10 days) associated with lower sympathetic activity (8, 24), followed by elevated heart rate in the latter stage of exposure (more than 10 days) due to withdrawal of vagal tone (9). The reduced heart rate observed during 4 days of HDT in the present study reflected an early stage of adaptation.

Peripheral α_1-adrenergic responsiveness appeared unaltered by 4 days of HDT in the present experiment as evidenced by no change in TPR to graded infusions of phenylephrine. This finding was similar to that in humans exposed to 14 days of HDT (8), suggesting that microgravity exposure does not alter α_1-adrenoreceptor responsiveness. In contrast, the slope of the stimulus-response relationship between graded isoproterenol infusion and heart rate was increased during HDT compared with control condition, a finding consistent with the human response of increased β_1-receptor responsiveness (8). Increased heart rate responsiveness to isoproterenol with exposure to HDT in our animals supports the notion that cephalid fluid shifts similar to those observed in microgravity induced a hypoadrenergic state with low neuronal release of norepinephrine (8, 24).

Our results from the LBNP experiments concur with previous data in humans that orthostatic hypotension and intolerance following HDT were associated with reduced stroke volume and cardiac output despite reflex elevations in heart rate and peripheral resistance. Direct invasive measurements of hemodynamic responses during graded orthostatic challenges in the present study extended previous experiments and provided new insight into underlying mechanisms. Data from ground-based human experiments suggested that lower filling pressure (lower CVP) appeared to represent a primary mechanism for reduced stroke volume during LBNP following HDT (6). We also observed that five of the six animals in the present study were unable to complete the LBNP protocol following HDT as a consequence of being orthostatically compromised by reduced filling pressures and stroke volumes. However, the maintenance of left ventricle area during HDT in the face of reduced CVP in the present study may indicate that increased cardiac compliance protects cardiac filling under conditions of lower filling pressure. Importantly, left ventricular dP/dt during LBNP was dramatically reduced by HDT in our animals, suggesting that the reduction in stroke volume and mean arterial pressure associated with lower orthostatic performance may be caused by reduced cardiac contractility rather than filling. We are unaware of any previous data that demonstrate a reduction in ventricular contractility during an orthostatic challenge following adaptation to actual or simulated microgravity. Therefore, our results may be the first to demonstrate that deterioration of myocardial performance may be an important consequence of cardiovascular adaptation to microgravity conditions in the absence of prophylactic interventions.

Our experimental conditions were not without potential limitations. Varying measurement error could have been associated with using EsP pressure as an index of pleural pressure due to measurement in the supine posture, potential compression of the trachea, and respiratory effects (10, 21, 22); TEE probe-induced vagal stimulation; and partial occlusion of the lower extremities caused by the LBNP inflatable skirt. Sedation by ketamine was necessary to allow TEE, CVP, and EsP measurements during baseline and LBNP tests and provide critical data for assessing cardiac function and hemodynamic responses. Although ketamine has been shown to increase blood pressure and affect other hemodynamic responses (28), care was taken to control

the time and dosage when ketamine was administered during our experiments. There was no evidence that this occurred. Also, anesthesia was identical for both HDT and control measurements, making it unlikely that this drug affected the results. A major strength of this study was in its experimental design in which each subject received both HDT and control conditions in random, counterbalanced order and all measurements were recorded in the 0° prone posture. This approach makes it unlikely that any differences observed under our experimental conditions could be explained by factors other than HDT.

In summary, 4 days of exposure to 10° HDT resulted in a reduction in CVP that was associated with increased left ventricular compliance rather than altered intrathoracic pressure. Reduced stroke volume, cardiac output, and mean arterial pressure during LBNP were associated with reduced myocardial contractility rather than reduced left ventricular preload.

Perspectives

It has generally been thought that cardiovascular adaptation to bed rest and spaceflight could be characterized by decreased left ventricular filling pressure, which led to decreased stroke volume, decreased cardiac output, and decreased orthostatic tolerance. Invasive measurements in spaceflight provided the surprising finding that CVP was low despite maintained or increased left ventricular diastolic volume and stroke volume. An increase in left ventricular compliance was postulated, although left ventricular compliance and intrathoracic pressures were not measured. Collection of additional invasive data during bed rest confirms the hypothesis of Buckey and co-workers (2) regarding increased compliance, but also provides new information on the adaptive process. The present study shows that two factors are operating simultaneously: increased left ventricular compliance, which would be expected to increase stroke volume, but apparently not enough to offset the decrease caused by reduced left ventricular contractility. Whether a generalized hypoadrenergic state explains both conditions is not clear. It seems more likely that increased left ventricular compliance is related to decreased pulmonary compression of the heart, whereas the decreased contractility results from a hypoadrenergic state. In either case, it is clear that reduced preload is not a sufficient explanation for the observed orthostatic intolerance in these settings and that more attention must be focused on neurohumoral mechanisms of circulatory control as well as the role pulmonary blood volume distribution in determining left ventricular compliance. The findings are highly relevant, not only to astronauts returning from spaceflight, but to hospitalized patients subjected to bed rest.

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